Abstract #10616

Use of the DEPArray platform to detect, isolate, and molecularly characterize pure tumor cells from peripheral blood samples enriched using the CellSearch® system

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Use of the DEPArray platform to detect, isolate, and molecularly characterize pure tumor cells from peripheral blood samples enriched using the CellSearch® system.

**Background:** Circulating tumor cells (CTC) offer the potential for serially monitoring the molecular profile of a tumor. However, enrichment techniques provide a level of purity problematic for most molecular analysis methods, and do not readily provide for analysis of tumor cell heterogeneity. We evaluate the use of DEPArray® (Silicon Biosystems), an automated system enabling image-based cell sorting with single-cell resolution, for CTC isolation and characterization from enriched blood samples.

**Methods:** Experiments were carried out with healthy-donor blood (HB) collected in CellSave tubes, spiked with tumor cells (TC) and enriched on Veridex’s AutoPrep with the CellSearch® Epithelial Cell Kit. DEPArray® system was used for detection and multiple recoveries of single TCs (or control WBCs) and 5 cell batches. A comparison of blind enumeration results with Veridex’s CellTracks Analyzer II® (CTAII) was carried out on replicate samples.

**Results:** No TCs were detected among negative controls (n=10). TC count (normalized to sample volume analyzed) was compared sample-wise for each replicate (n=20). DEPArray® and CTAII counts were, on average, 100% (standard deviation = 52%). Enriched mixtures of Her2+ and Her2- TCs spiked in HB samples (n=5), were sorted by DEPArray® and recovered into separate tubes. By phenotypical re-analysis no Her2- cells were detected among the Her2+ cell fraction and vice versa, neither were donor WBCs found (100% purity). KRAS-mutated, A549 cells spiked in HB were enriched and loaded on DEPArray®. Individual fractions containing either 1-5 tumor cells or donor WBCs were sorted. Whole Genome Amplification ( Amplicom, silicon Biosystems), KRAS specific gene amplification and Capillary Electrophoresis sequencing were carried out. TCs successfully amplified showed only mutated KRAS (WBCs were only wild-type). 100% purity, eliminating all white blood cells (WBC), in the isolation of a mixed population of tumor cell lines downstream of CellSearch® enrichment. This enabled molecular profiling of pure tumor cells from whole blood spiked tumor cell lines. Detection of molecular heterogeneity of tumor cells is demonstrated through KRAS sequencing.

**Conclusions:** DEPArray® achieved 100% purity, eliminating all white blood cells (WBC), in the isolation of a mixed population of tumor cell lines downstream of CellSearch® enrichment. This enabled molecular profiling of pure tumor cells from whole blood spiked tumor cell lines. Detection of molecular heterogeneity of tumor cells is demonstrated through KRAS sequencing.

**Tumor Cell Detection**

**Cell Separation**

**Cell Molecular Characterization**

**Summary of results**

Cells prepared in Veridex central lab, PA (V-HV); counted performed in blind by remote labs: Veridex Enschede, NL (V-EU) and Silicon Biosystems (SB) with DEPArray. Negative controls (only Healthy donor Blood, n=10), were negative in all platforms (data not shown). For spiking experiments (n=20, Table 1) of TCs (SKBr3), a normalization coefficient (9.26/15=61.7%) is used on Veridex counts to take into account volume effectively analyzed by DEPArray (Main Chamber volume). DEPArray/CTAII remote count ratio = SB/V-EU=100%, o=52%. Average number of TC in remote labs ([SB+V-EU]/2)=(V-HV)≈80% due to shipping loss, o=16%. (cor(SB/V-EU) due to negative correlation from sample splitting after pooling).

**Table 1**

<table>
<thead>
<tr>
<th>TC</th>
<th>Her2+</th>
<th>Her2-</th>
<th>Purity</th>
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<tr>
<td>1</td>
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</tr>
<tr>
<td>5</td>
<td>16</td>
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</table>

**Figure**

**DEPArray™ Cell Browser screenshot**

**QMS17 Quadrupole Magnetic Concentration**

**CellTracks Analyzer II® Images**

**Cell Separation**

**Experiment work-flow**

**Cell Molecular Characterization**

**Molecular Analysis Results**

**Summary**

Across four Spiking experiments of viable (>90%) KRAS mutated cell lines (SW480 n=2, A549 n=1, mix SW480/A549 n=1) in HB, multiple recoveries (range 12-21 per experiment) of individual cells (n=5), 5 cell batches (n=5), or negative controls (n=8) were carried out. Amplicom® WGA Kit (Silicon Biosystems) products from each tube were DNA-fingerprinted (home-brewed 11 loci multiplex reaction, to confirm cell presence, identity and purity) and KRAS gene-specific amplification products were sequenced (on ABI 3730XL). In 91% (51/56) of single cell recoveries, cell presence was confirmed. The 5 tubes with no signal from STR and KRAS suggests that the cell has been removed during surfactant removal before WGA. All successfully amplified cells matched 100% KRAS mutational status and DNA fingerprint, no signals was detected in negative controls recoveries (buffer only). In the mixed tumor cells experiment, different KRAS mutations (and DNA fingerprints) were detected in different tumor cell recoveries, reflecting cell heterogeneity.